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METHOD DEVELOPMENT, VALIDATION, AND STABILITY INDICATING STUDIES OF OLMESARTAN MEDOXOMIL AND HYDROCHLORTHIAZIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY UV-SPECTROSCOPY

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Abstract:

Development of UV method for simultaneous estimation of Olmesartan Medoxomil development was done by Q-Absorbance ratio method and area under curve method and stability indicating studies using methanol as solvent. Most of the studies are not well validated and not cross validated by other methodology. Here we have made an attempt to develop a simple, specific, accurate, precise and reproducible method for the simultaneous estimation of hydrochlorothiazide and OLM in combined dosage form by UV spectrophotometric method, the method includes area under curve method (Method I) and Q- absorbance Ratio method (Method II). The wavelengths are 243 nm and 272 nm λ_{max} of both the drugs were selected for Method I, and for Q- absorbance Ratio method (Method II) 250 nm an isoabsorptive wavelength and 272 nm were selected for estimation of Olmesartan Medoxomil and Hydrochlorothiazide respectively and The two drugs follow Beer's law over the concentration range of 1-6 µg/ml.

The % recoveries of the both the drugs were found to be nearly 100 % representing the accuracy of the proposed methods. LOD and LOQ values of OLM was found to be 0.400,0.403,0.407,0.400,0.403,0.407 at different wavelengths 272nm, 250nm, 242nm and LOD LOQ values of HTZ were found to be 0.135, 0.133, 0.182, 0.410, 0.405, 0.550 at 272nm, 250nm, 242nm.

Validation of the proposed methods was carried out for its accuracy, precision, specificity and ruggedness according to ICH guidelines. The proposed methods successfully applied in routine work for determination of Olmesartan medoxomil and hydrochlorothiazide in combined dosage form.

Key words: Hydrochlorothiazide (HTZ), Olmesartan Medoxomil (OLM), Area undercurve, Q-absorbance ratio method, Stability studies.

Introduction:

Olmesartan medoxomil was an anti- hypertension drug chemically named as (5-Methyl-2-oxo-1,3-dioxol-4-yl) methyl5-(2-hydroxypropan-2-yl)-2-propyl-3-[4-[2-(2H-tetrazol-5-yl) phenyl] methyl] imidazole-4-carboxylate. OLM is one of several angiotensin II receptors blocking agents. OLM has been shown to have a longer half -life and a greater effect on systolic blood pressure than other ARB agents, making it widely prescribed for the management of hypertension. OLM is a inactive ester prodrug.

Hydrochlorthiazide was a first line diuretic drug. Chemically: 6-Chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide. It belongs to thiazides class of diuritics.it reduces blood volume by acting on kidneys to reduce sodium reabsorption in the distal convoluted tubule. Thiazides increases the reabsorption of calcium. It is believed to lower peripheral vascular resistance.

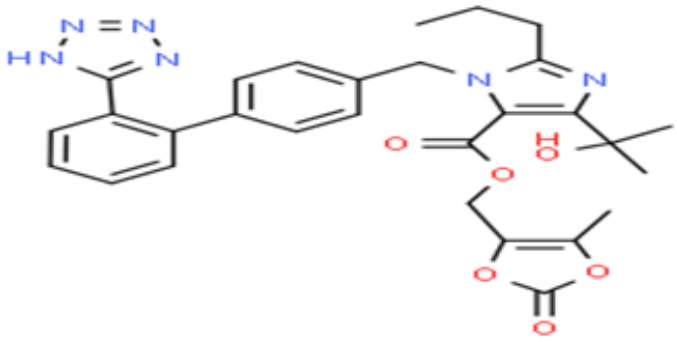

Figure-1: Structure of Olmesartan Medoxomil (OLM).

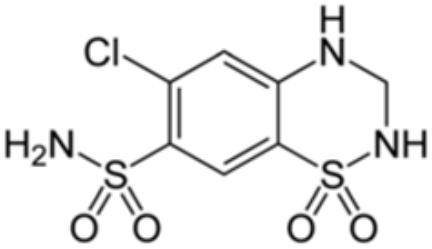

Figure-2: Structure of Hydrochlorthiazide (HTZ).

Materials and Methods [1-7]:

Chemicals and reagents:

Olmesartan Medoxomil and Hydrochlorthiazide procured from the KP laboratories. Commercial pharmaceutical preparation Olmesartan-Hydrochlorthiazide, manufactured by INTA pharmaceuticals,

containing 10mg of Olmesartan and 20mg of Hydroclorthazide was collected from local market, methanol analytical grade was procured from Quietens India Pvt Ltd**.**

Instrumentation:

The proposed methanol was carried on a shimadzu UV-Visible Spectrophotometer (UV-1800 series). All weighing was done on Digital balance (Shimadzu). A fast clean Ultra sonicator was used for degassing the solvent.

Selection of Solvents: On the basis of the solubility studies Methanol was selected as solvent for method development.

UV-Spectroscopy:

Preparation of Standard Solutions:

Weigh accurately 10mg of OLM and HTZ separately into a 100ml volumetric flask, add 10ml of solvent and shake well to dissolve the drug completely. Made up the volume to 100ml with solvent to get 100µg/ml of both OLM and HTZ.

Preparation of Sample Solution:

20 Tablets were taken, crushed to fine powder. An accurately weigh powder sample equivalent to 10mg of Olmesartan medoxomil powder as weighed and transferred to 100ml volumetric flask, dissolved in sufficient solvent and filtered through whatmann filter paper. The filtrate was made up to volume of 100ml with solvent get 100µg/ml of both OLM and HTZ.

Determination of λ_{max}:

Standard solutions of OLM and HTZ were prepared and scanned in UV- spectrophotometer in the range of 200-400nm to determine the λ_{max} of each drug λ_{max} of OLM and HCZ were found to be 250nm and 272nm respectively.

Method Development [8-12]**:**

1. Q-Absorbance ratio method: According to Q-absorption ratio method, use the ratio of absorption at two selected wavelengths. One is at iso-absorptive point and other being the λmax of one of the two components. Calculate the concentrations of two components by using the equation

$$C_x = \{(Q_m-Q_y)/ (Q_x-Q_y)\}* (A_1/ax_1)$$

$C_y = \{(Q_m-Q_x)/ (Q_y-Q_x)\}* (A_1/ay_1)$

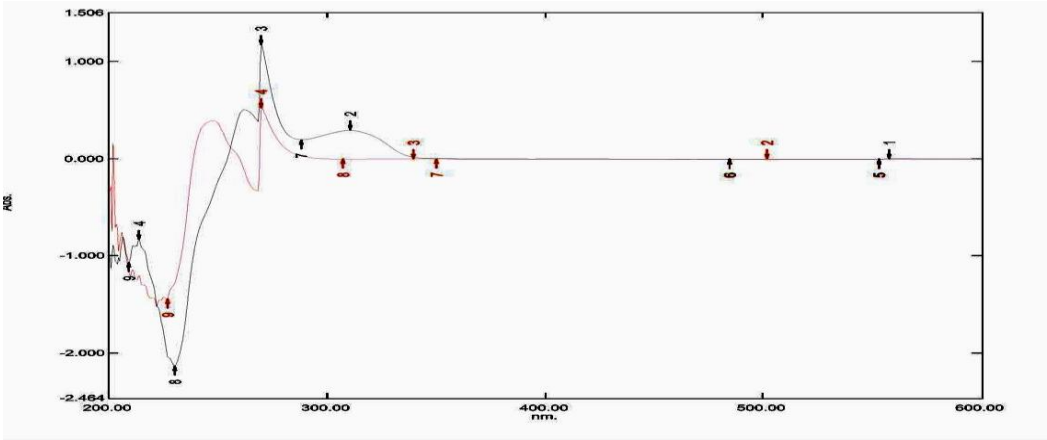

Figure-3: Overlay spectrum of OLM and HTZ

2. Area Under the Curve Method:

OLM and HTZ were scanned between 200-400nm and found 243nm for OLM and 272nm for HTZ as λmax for estimation using area undercurve method. Aliquotes of 1-6 µg/ml solutions was prepared using methanol as solvent and measured absorbance of drugs at λmax.

$$C^M = X^N_{\lambda1-\lambda2} AUC_{\lambda3-\lambda4} - X^N_{\lambda3-\lambda4} AUC_{\lambda1-\lambda2} / X^N_{\lambda1-\lambda2} = X^M_{\lambda3-\lambda4} - X^N_{\lambda3-\lambda4} X^M_{\lambda1-\lambda2}$$

$$C^N = X^M_{\lambda1-\lambda2} AUC_{\lambda3-\lambda4} - X^M_{\lambda3-\lambda4} AUC_{\lambda1-\lambda2} / X^N_{\lambda1-\lambda2} = X^M_{\lambda3-\lambda4} - X^N_{\lambda3-\lambda4} X^M_{\lambda1-\lambda2}$$

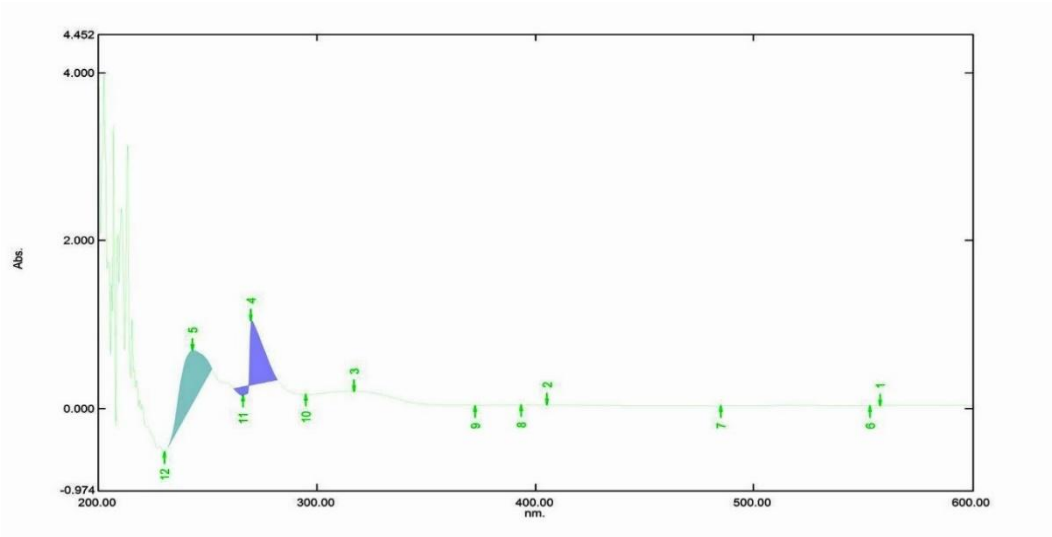

Figure-4: Area under curve of OLM and HTZ.

Validation of the Method [13-14]:

UV-VIS Spectroscopic method was validated according to International Conference on Harmonization (ICH) guidelines. The following characteristics were considered for validation: linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ).

Linearity: The methods were validated according to International conference on Harmonization guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for each analyte. Calibration curves were generated with appropriate volumes of working standard solutions for UV and with the range of 1-5 respectively. The linearity was evaluated by the least square regression method using unweighed data

Accuracy and Precision: The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) and reported as %RSD for a statistically significant number of replicate measurements. The intermediate precision was studied by comparing the assay on 3 different days and the results documented as standard deviation and %RSD. Accuracy is the percent of analyte recovered by assay from a known added amount. For the measurement of accuracy data from nine determinations over three concentration levels covering the specified range were determined.

Robustness: Robustness of the method was determined by making slight changes in the chromatographic conditions, such as composition mobile phase ratio and wavelength and flow rate.

LOD and LOQ: Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentration of OLM and HTZ. The LOD and LOQ values were calculated by using the following formula:

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where σ = The standard deviation of the response

S = Slope of calibration curve.

Results and Discussion:

Table-1: Q-Absorbance ratio method values of OLM & HTZ.

Concentration (µg/mL)	OLM 250nm	OLM 272nm	HTZ 250nm	HTZ 272nm
1	0.12	0.105	0.14	0.16
2	0.229	0.231	0.259	0.347
3	0.301	0.321	0.364	0.497
4	0.432	0.404	0.479	0.654
5	0.543	0.557	0.581	0.797

Table-2: Area under curve of OLM and HTZ.

Concentration(µg/mL)	OLM 243nm	HTZ 272nm
1	0.05456	0.07762
2	0.11942	0.16241
3	0.19421	0.26274
4	0.25421	0.31421
5	0.29940	0.39781
6	0.35761	0.465432
Mean	0.1536	0.2399
SD	0.1311	0.1695

Linearity: A series of solutions in the concentration range of 1-6µg/mL of OLM and 1-6µg/mL of HTZ from the stock solutions were prepared. These solutions were scanned in the range of 200-400 nm and the absorbance was noted at the λ_{max} of each drug (272 nm) for HTZ and 242 nm for OLM.

Table-3:Linearity of HTZ.

Concentration(µg/ml)	Absorbance
1	0.07762
2	0.16241
3	0.26274
4	0.31421
5	0.39781
6	0.46532

Table-4: Linearity of OLM.

Concentration(µg\ml)	Absorbance
1	0.0865
2	0.1625
3	0.234
4	0.3185
5	0.410
6	0.482

Table-5: Intraday precision.

concentration	272nm	250nm	242nm	272nm	258nm	242nm
3	0.995	0.225	0.678	0.472	0.475	0.389
3	0.996	0.223	0.678	0.471	0.48	0.396
3	0.997	0.222	0.677	0.470	0.486	0.399
3	0.998	0.219	0.675	0.473	0.483	0.402
3	0.999	0.217	0.675	0.573	0.484	0.403
Mean	0.997	0.221	0.677	0.472	0.484	0.398
SD	0.0014	0.0029	0.012	0.0012	0.0058	0.0050
%RSD	0.1418	1.2914	0.1723	0.2472	1.1905	1.2659

Table-6:**Inter day precision.**

Concentration	272nm	250nm	242nm	272nm	250nm	242nm
3	0.994	0.226	0.672	0.473	0.473	0.386
3	0.995	0.225	0.673	0.472	0.474	0.387
3	0.996	0.221	0.674	0.471	0.475	0.388
3	0.997	0.221	0.675	0.473	0.476	0.389
3	0.998	0.219	0.676	0.472	0.477	0.402
Mean	0.996	0.222	0.674	0.473	0.475	0.390
SD	0.0014	0.0027	0.0014	0.0010	0.0014	0.059
%RSD	0.1420	0.0894	0.2747	0.1925	0.1935	0.1574

Table-7: Robustness of OLM.

Drug	Changes in wavelengths	Absorbance
OLM	243	0.0866
	244	0.0867
	245	0.0868
	246	0.0869
	247	0.0869

Table-8: Robustness of HTZ.

Drug	Changes in wavelengths(±1nm)	Absorbance
HTZ	273	0.07763
	274	0.07764
	275	0.07765
	276	0.07766
	277	0.07767

Table-9: LOD and LOQ of OLM.

Parameter	Olmesartan		
	Methods -A		**Method -B**
	272nm	242nm	252nm
LOD	0.146	0.136	0.201
LOQ	0.422	0.488	0.407

Table-10: LOD and LOQ of HTZ.

Parameter	Hydrochlorthiazide		
	Methods -A		**Method -B**
	272nm	242nm	252nm
LOD	0.135	0.133	0.182
LOQ	0.410	0.405	0.555

Accuracy:

Table-11: Accuracy of OLM.

Methods	Amount taken	Amount found	%Recovery
Method A	50	**0.**139	99.7
	100	**0.**147	99.8
	150	**0.**235	100.1
Method B	50	0.142	99**.9**
	100	0.145	99.9
	150	0.232	100.2

Table-12: Accuracy of HTZ.

Methods	Amount taken	Amount found	%Recovery
Method A	50	0.140	99.5
	100	0.148	99.7
	150	0.230	100.2
Method B	50	0.145	99.4
	100	0.147	100.3
	150	0.235	100.1

Table-13: Forced degradation studies.

Stress Degradation Condition	Area Under Curve	%Degradation	Active drug process after degradation (%)
Standard drug	3.866	0	100
Acid Degradation	1.723	64.26732673	57.73267327
Base degradation	0.832	79.9669967	30.0330033
Oxidative degradation	0.25	61.50825083	44.49174917
Photo stability degradation	0.26	85.80858086	14.19141914

Conclusion:

The proposed UV Spectrophotometric methods are simple, fast, sensitive, accurate, precise, less time-consuming and economic. All the parameters were found to be within limits according ICH guidelines. Hence stability indicating studies have been developed for the estimation of Olmesartan and Hydrochlorthiazide.The use of Chemistry methods has proved to be a smart strategy to provide both environmental and economic benefits. Hence these methods can be used for the routine analysis of Olmesartan and Hydrochlorthiazide

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